

TNF- α induced apoptosis of osteocytes is inhibited by mechanical loading

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Bone has the capacity to alter its mass and structure to its mechanical environment. It is generally accepted that osteocytes are the mechanosensitive bone cells regulating the remodeling process. When bone is loaded, extracellular fluid is squeezed through the thin layer of non-mineralized matrix surrounding the osteocytes toward the bone surface. This flow produces fluid shear stress at the osteocyte membrane that activates the osteocyte. Absence of loading results in local stasis of extracellular fluid in the canalicular network and induces regulated osteocyte death, i.e. apoptosis. It has been observed that apoptotic osteocytes are often in contact with osteoclasts (Bronckers et al., *J. Bone Miner Res* 1996) and therefore we suggest that osteocyte apoptosis plays a key role in the bone remodeling event. Tumor Necrosis Factor- α is a pro-inflammatory catabolic cytokine with apoptotic potency. In bone, it stimulates osteoclastogenesis and inhibits osteoblast function. Elevated levels are found in bone diseases like osteoporosis and periodontitis. Here we investigated if TNF- α affected apoptosis in osteocytes (OCY), osteoblasts (OB) and periosteal fibroblasts (PF), and whether mechanical loading could affect this process.

OCY, OB, and PF were isolated from fetal chicken calvaria via enzymatic digestion. Separation of the periosteum from calvaria occurred to obtain PF. mAb 7.3, an antibody specific for OCY, was used to separate OCY from OB by immunomagnetic separation. Apoptosis was induced by 10 ng/ml TNF- α for 16 h. Subsequently, cells were treated for 1 h with a pulsating fluid flow shear stress (PFF; 0.70 ± 0.30 Pa, 5 Hz) or were kept under static conditions. After a 24 h post-incubation period, apoptosis was assessed as caspase 3/7 activity using the Caspase-Glo 3/7 Assay (Promega).

TNF- α increased the caspase 3/7 activity of both OCY and OB (n=4) by approximately 2-fold compared to untreated cultures, but did not affect caspase 3/7 activity of PF (n=3) (figure 1). Treatment with PFF reduced the TNF- α -induced caspase 3/7 activity in OCY (n=8) compared to static cultures by 25%. No effect of fluid flow was seen in cultures of OB (n=6) and PF (n=7) (figure 2).

Our results demonstrate that TNF- α induces apoptosis in both OCY and OB. PF, however, failed to undergo apoptosis in response to TNF- α . This suggests that TNF- α affects apoptosis of mature bone cells such as OCY and OB, but not cells of the PF population, containing immature bone cells and osteoprogenitor cells. Furthermore, the study demonstrates that mechanical loading by PFF inhibits TNF- α induced apoptosis of OCY. In OB and PF, however, apoptosis could not be inhibited by PFF. This suggests that osteocyte survival, or escape from the apoptotic effect of TNF- α , is quite sensitive to fluid shear stress. The absence of shear stress leads to osteocyte apoptosis that may result in osteoclast recruitment and subsequent bone resorption.

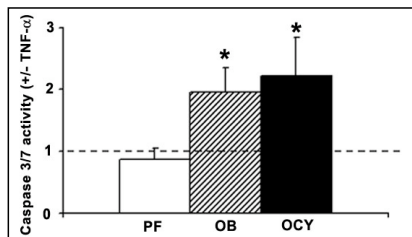


Figure 1: Effect of TNF- α on caspase 3/7 activity

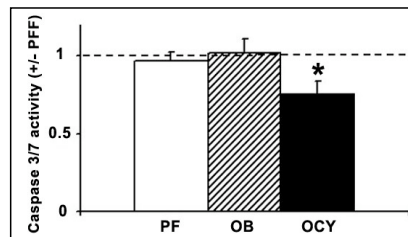


Figure 2: Effect of PFF on caspase 3/7 activity